

1 **Supplementary Results**

2 Table S1: Meta data for the 24 phenotypically extreme cows including ID, age (in years) their
3 classification as wither CHDL case or control based on CHDL scoring, whether a foot biopsy
4 sample was taken and the average CHDL score (calculated based on the CHDL score given at
5 each of the four lactation stages over the study period, detailed in methods).

Cow ID	Age (years)	CHDL case/control	Foot biopsy taken	Averages CHDL core
2546	4	Case	Yes	0.625
2344	5	Control	Yes	0.188
2603	4	Case	Yes	0.469
897	7	Case	Yes	0.688
2633	5	Control	NO	0.042
2685	5	Control	NO	0.063
2338	5	Case	Yes	0.938
2330	5	Control	NO	0.156
2345	5	Control	Yes	0.063
2353	5	Control	Yes	0.188
2179	7	Control	NO	0.031
2911	4	Control	NO	0.083
2381	5	Case	Yes	0.813
2402	5	Control	Yes	0.219
2606	4	Case	NO	0.375
2319	5	Control	Yes	0.125
2152	6	Case	Yes	0.708
2628	4	Control	Yes	0.156
2391	5	Case	Yes	0.458
3013	4	Case	NO	0.688
2376	6	Case	NO	0.531
2829	5	Case	NO	0.417
2812	5	Case	NO	0.417
2524	4	Control	Yes	0.156

7 *Table S2: The top ten markers (by lowest p value) associated with CHDL as a combined trait*
8 *detailing genomic position, lambda corrected p-value and the nearest gene to the genomic*
9 *location.*

Chromosome	Position	p_value	Nearest gene ID	Nearest gene name
5	87262266	1.39E-05	ENSBTAG00000005833	ETNK1
1	147338727	2.53E-05	ENSBTAG00000004742	RUNX1
15	5234380	2.74E-05	ENSBTAG00000019309	DYNC2H1
1	147241898	3.44E-05	ENSBTAG00000004742	RUNX1
3	30167683	3.79E-05	ENSBTAG00000027172	Novel cattle gene
14	78522424	4.47E-05	ENSBTAG00000046821	Novel cattle gene
5	88079689	5.21E-05	ENSBTAG00000026611	Novel cattle gene
3	88991871	5.70E-05	ENSBTAG00000013802	DAB1
10	61837266	7.41E-05	ENSBTAG00000002278	FBN1
23	43909068	9.08E-05	ENSBTAG00000012039	PHACTR1

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11 *Table S3: The top 10 genomic windows explaining the most genetic variance. Genomic window*
12 *name indicates the genomic position (chromosome: start bp – end bp).*

Genomic window	% genetic variance explained
BTA3:88750416-88750416bp	0.54
BTA3:88770660-88770660bp	0.54
BTA3:88796395-88796395bp	0.49
BTA10:80785147-80785147bp	0.48
BTA3:88821997-88821997bp	0.47
BTA10:80790117-80790117bp	0.47
BTA10:80740519-80740519bp	0.47
BTA10:80762670-80762670bp	0.47
BTA10:80818350-80818350bp	0.46
BTA10:80799562-80799562bp	0.45

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14 *Table S4: Chi square results of the distribution of SNPs in the top 100 GWAS windows identified*
15 *by WGS. Results of the Chi square comparisons ($p \leq 0.10$) of the frequency of SNP variants*
16 *between case and control in the top 100 GWAS windows. Table shows the SNP position and*
17 *associated gene, the Chi-squared statistic, degrees of freedom and p value for each SNP along*
18 *with the assigned consequence and impact by VEP.*

Gene name	SNP	X ²	df	P value	Consequence	IMPACT
ACTR3B	BovChr4Pos115184022	9.17	3	0.027	3_prime_UTR_variant	MODIFIER
ACTR3B	BovChr4Pos115184077	9.17	3	0.027	3_prime_UTR_variant	MODIFIER
XRCC2	BovChr4Pos115031057	8.77	3	0.033	missense_variant	MODERATE
ACTR3B	BovChr4Pos115181797	8.25	3	0.041	synonymous_variant	LOW
XRCC2	BovChr4Pos115031007	7.88	3	0.049	missense_variant	MODERATE
SLC29A3	BovChr28Pos27453921	7.47	3	0.058	5_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141656323	5.57	2	0.062	synonymous_variant	LOW
MX2	BovChr1Pos141656417	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141656685	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141656957	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141657878	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141658152	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141658169	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
MX2	BovChr1Pos141658170	5.57	2	0.062	3_prime_UTR_variant	MODIFIER
PRKAA2	BovChr3Pos89549747	7.24	3	0.065	upstream_gene_variant	MODIFIER
LRP8	BovChr3Pos92976498	5.47	2	0.065	synonymous_variant	LOW
H3-3B	BovChr19Pos55821471	7.01	3	0.071	5_prime_UTR_variant	MODIFIER
FYB2	BovChr3Pos89409465	6.57	3	0.087	synonymous_variant	LOW
CCDC17 7	BovChr10Pos81312514	6.55	3	0.088	synonymous_variant	LOW
EVPL	BovChr19Pos55642058	4.85	2	0.089	synonymous_variant	LOW
EVPL	BovChr19Pos55643109	4.85	2	0.089	synonymous_variant	LOW
EVPL	BovChr19Pos55643198	4.85	2	0.089	missense_variant	MODERATE
EVPL	BovChr19Pos55643415	4.85	2	0.089	synonymous_variant	LOW
EVPL	BovChr19Pos55643499	4.85	2	0.089	synonymous_variant	LOW
EVPL	BovChr19Pos55643604	4.85	2	0.089	synonymous_variant	LOW
EVPL	BovChr19Pos55643616	4.85	2	0.089	synonymous_variant	LOW
CDK3	BovChr19Pos55645229	4.85	2	0.089	3_prime_UTR_variant	MODIFIER
RECQL5	BovChr19Pos55943421	4.85	2	0.089	splice_acceptor_variant	HIGH
RECQL5	BovChr19Pos55943422	4.85	2	0.089	splice_acceptor_variant	HIGH
ACTR3B	BovChr4Pos115133532	6.46	3	0.091	5_prime_UTR_variant	MODIFIER
SUSD6	BovChr10Pos81361127	4.61	2	0.100	5_prime_UTR_variant	MODIFIER

20 *Table S5: Differentially expressed genes between CHDL case and Control samples of bovine peripheral blood lymphocytes of dairy cows. Genes were*
21 *considered for Gene Set Enrichment Analysis if they had a log2 fold change greater than or equal to 1 or less than or equal to -1, an unadjusted p-value*
22 *of 0.05 or lower and baseMean greater than or equal to 100.*

Gene ID	Gene name	Strand	Gene biotype	baseMean	log2fc	Raw <i>P</i> - value	FDR
<i>ENSBTAG00000025260</i>	<i>ENSBTAG00000025260</i>	-	protein_coding	35.80712	24.53424	4.84E-11	0.00
<i>ENSBTAG00000048406</i>	<i>ENSBTAG00000048406</i>	-	lncRNA	1.050457	23.96103	1.51E-10	0.00
<i>ENSBTAG00000002928</i>	<i>AMTN</i>	+	protein_coding	20.42442	22.80508	1.07E-09	0.00
<i>ENSBTAG00000054927</i>	<i>KRTAP3-3</i>	-	protein_coding	12.9061	22.39195	2.18E-09	0.00
<i>ENSBTAG00000051867</i>	<i>ENSBTAG00000051867</i>	-	protein_coding	5.237257	21.95632	4.45E-09	0.00
<i>ENSBTAG00000052202</i>	<i>KRTAP27-1</i>	+	protein_coding	6.172482	22.17438	3.13E-09	0.00
<i>ENSBTAG00000052798</i>	<i>ENSBTAG00000052798</i>	-	protein_coding	10.05845	21.8952	4.90E-09	0.00
<i>ENSBTAG00000054136</i>	<i>ENSBTAG00000054136</i>	-	protein_coding	10.05845	21.8952	4.90E-09	0.00
<i>ENSBTAG00000045125</i>	<i>ENSBTAG00000045125</i>	-	snoRNA	0.214995	-20.9025	3.07E-08	0.00
<i>ENSBTAG00000038893</i>	<i>ENSBTAG00000038893</i>	-	protein_coding	1.148648	20.73576	3.44E-08	0.00
<i>ENSBTAG00000051801</i>	<i>ENSBTAG00000051801</i>	+	protein_coding	5.130092	20.51375	5.61E-08	0.00
<i>ENSBTAG00000040416</i>	<i>5_8S_rRNA</i>	-	rRNA	0.14333	-18.6334	8.01E-07	0.00
<i>ENSBTAG00000043565</i>	<i>ENSBTAG00000043565</i>	-	Mt_tRNA	0.14333	-18.6334	8.01E-07	0.00
<i>ENSBTAG00000043760</i>	<i>5S_rRNA</i>	-	rRNA	0.258558	17.99013	1.93E-06	0.00
<i>ENSBTAG00000053198</i>	<i>ENSBTAG00000053198</i>	-	protein_coding	781.3787	8.243587	4.96E-06	0.01
<i>ENSBTAG00000038497</i>	<i>ENSBTAG00000038497</i>	-	protein_coding	9.113257	16.53646	9.93E-06	0.02

<i>ENSBTAG00000011182</i>	<i>PDLIM1</i>	-	protein_coding	303.8617	2.137501	2.01E-05	0.03
<i>ENSBTAG00000019349</i>	<i>SLC6A4</i>	-	protein_coding	128.2617	1.746067	4.53E-05	0.06
<i>ENSBTAG00000019297</i>	<i>GFRA1</i>	-	protein_coding	11.61218	6.462495	5.56E-05	0.07
<i>ENSBTAG00000042411</i>	<i>U6</i>	+	snRNA	29.78555	4.444743	6.95E-05	0.09

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25 Table S6: Gene Set Enrichment Analysis (GSEA) results based on the differential expression analysis of the peripheral blood lymphocytes
 26 transcriptome when CHDL Case samples compared to Control samples. The Table presents the significant biological processes (BP), Cellular
 27 Component (CC) and Molecular Function (MF) enriched among the differentially expressed genes. Columns include the Ontology and Gene Ontology
 28 ID, Ontology description, the number of genes in the set (setSize), the normalized enrichment score (NES), the FDR adjusted p-value, and the gene
 29 set names. Positive NES values represent enrichment in upregulated genes, while negative NES values indicate enrichment in downregulated genes.

ONTOLOGY	ID	Description	setSize	NES	FDR	Gene set enriched
BP	GO:0006807	nitrogen compound metabolic process	3	1.85	0.001	ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000011182
BP	GO:0048522	positive regulation of cellular process	6	1.87	0.005	ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000011182/ENSBTAG00000015434/ENSBTAG00000019349
BP	GO:0050794	regulation of cellular process	9	1.80	0.009	ENSBTAG00000002278/ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000011182/ENSBTAG00000015434/ENSBTAG00000019349
BP	GO:0048519	negative regulation of biological process	3	1.68	0.013	ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000019349
BP	GO:0051239	regulation of multicellular organismal process	3	1.68	0.013	ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000019349
BP	GO:0065007	biological regulation	13	1.80	0.014	ENSBTAG00000002278/ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000011182/ENSBTAG00000012265/ENSBTAG00000015434/ENSBTAG00000019349/ENSBTAG00000006035/ENSBTAG00000014614
BP	GO:0044238	primary metabolic process	4	1.74	0.014	ENSBTAG00000014824/ENSBTAG00000047330/ENSBTAG00000011182

BP	GO:0048518	positive regulation of biological process	7	1.72	0.019	ENSBTAG00000014824/ENSBTAG00000047330/ ENSBTAG00000011182/ENSBTAG00000015434/ ENSBTAG00000019349/ENSBTAG00000014614
BP	GO:0009987	cellular process	17	1.78	0.021	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000009959/ENSBTAG00000014614/ ENSBTAG00000037644/ENSBTAG00000004948
BP	GO:0008150	biological_process	20	1.69	0.022	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000009959/ENSBTAG00000006035/ ENSBTAG00000014614/ENSBTAG00000037644/ ENSBTAG00000004948
BP	GO:0050789	regulation of biological process	10	1.69	0.023	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000015434/ENSBTAG00000019349
BP	GO:0009893	positive regulation of metabolic process	4	1.65	0.031	ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000019349/ENSBTAG00000014614
BP	GO:0010467	gene expression	4	1.65	0.031	ENSBTAG00000014824/ENSBTAG00000011182/ ENSBTAG00000019349/ENSBTAG00000014614
BP	GO:0019222	regulation of metabolic process	4	1.65	0.031	ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000019349/ENSBTAG00000014614
BP	GO:0043170	macromolecule metabolic process	4	1.65	0.031	ENSBTAG00000014824/ENSBTAG00000011182/ ENSBTAG00000019349/ENSBTAG00000014614

BP	GO:0008152	metabolic process	6	1.65	0.031	<i>ENSBTAG00000014824/ENSBTAG00000047330/ ENSBTAG00000011182/ENSBTAG00000019349/ ENSBTAG00000014614</i>
BP	GO:0071704	organic substance metabolic process	6	1.65	0.031	<i>ENSBTAG00000014824/ENSBTAG00000047330/ ENSBTAG00000011182/ENSBTAG00000019349/ ENSBTAG00000014614</i>
BP	GO:0065008	regulation of biological quality	6	1.64	0.034	<i>ENSBTAG00000047330/ENSBTAG00000012265/ ENSBTAG00000015434/ENSBTAG00000019349/ ENSBTAG00000014614</i>
BP	GO:0007165	signal transduction	5	1.65	0.037	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330</i>
BP	GO:0050896	response to stimulus	9	1.62	0.037	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000012265/ ENSBTAG00000019349/ENSBTAG00000014614/ ENSBTAG00000037644</i>
BP	GO:0007154	cell communication	6	1.62	0.041	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000017425</i>
BP	GO:0023052	signaling	6	1.62	0.041	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000017425</i>
CC	GO:0030312	external encapsulating structure	3	1.77	0.004	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000012265</i>
CC	GO:0031012	extracellular matrix	3	1.77	0.004	<i>ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000012265</i>
CC	GO:0030054	cell junction	3	1.68	0.013	<i>ENSBTAG00000047330/ENSBTAG00000017425/ ENSBTAG00000019349</i>
CC	GO:0070161	anchoring junction	3	1.68	0.013	<i>ENSBTAG00000047330/ENSBTAG00000017425/ ENSBTAG00000019349</i>

CC	GO:0005575	cellular_component	20	1.65	0.040	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000006035/ENSBTAG00000014614/ ENSBTAG00000013755/ENSBTAG00000004948/ ENSBTAG000000054533/ENSBTAG00000007434/ ENSBTAG00000013953/ENSBTAG00000020755/ ENSBTAG00000027126
CC	GO:0110165	cellular anatomical entity	20	1.65	0.040	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000006035/ENSBTAG00000014614/ ENSBTAG00000013755/ENSBTAG00000004948/ ENSBTAG000000054533/ENSBTAG00000007434/ ENSBTAG00000013953/ENSBTAG00000020755/ ENSBTAG00000027126
CC	GO:0005737	cytoplasm	12	1.63	0.045	ENSBTAG00000014824/ENSBTAG00000047330/ ENSBTAG00000011182/ENSBTAG00000012265/ ENSBTAG00000015434/ENSBTAG00000019349/ ENSBTAG00000006035/ENSBTAG00000014614/ ENSBTAG00000004948
MF	GO:0042802	identical protein binding	4	1.87	0.003	ENSBTAG00000047330/ENSBTAG00000012265/ ENSBTAG00000017425/ENSBTAG00000019349
MF	GO:0003674	molecular_function	21	1.82	0.012	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/

						ENSBTAG00000009959/ENSBTAG00000006035/ ENSBTAG00000014614/ENSBTAG00000013755/ ENSBTAG000000037644/ENSBTAG00000020638
MF	GO:0044877	protein-containing complex binding	3	1.68	0.013	ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000019349
MF	GO:0043167	ion binding	11	1.80	0.013	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000014614/ENSBTAG00000037644/ ENSBTAG00000020638
MF	GO:0005488	binding	15	1.78	0.017	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000047330/ENSBTAG00000011182/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349/ ENSBTAG00000014614/ENSBTAG00000037644/ ENSBTAG00000020638
MF	GO:0043169	cation binding	8	1.69	0.028	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000011182/ENSBTAG00000017425/ ENSBTAG00000019349/ENSBTAG00000037644/ ENSBTAG00000020638
MF	GO:0046872	metal ion binding	8	1.69	0.028	ENSBTAG00000002278/ENSBTAG00000014824/ ENSBTAG00000011182/ENSBTAG00000017425/ ENSBTAG00000019349/ENSBTAG00000037644/ ENSBTAG00000020638
MF	GO:0005515	protein binding	10	1.66	0.029	ENSBTAG00000002278/ENSBTAG00000047330/ ENSBTAG00000012265/ENSBTAG00000015434/ ENSBTAG00000017425/ENSBTAG00000019349

MF	GO:0005215	transporter activity	5	1.56	0.038	<i>ENSBTAG00000047330/ENSBTAG00000017425/ ENSBTAG00000019349/ENSBTAG00000009959</i>
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31 *Table S7: Gene ontology enrichment results summary by Revigo based on the differential expression analysis of the peripheral blood lymphocytes*
32 *transcriptome when CHDL Cases compared to Control samples. . Columns include log(pvalue) of GO enrichment of term, LogSize (the Log10(number*
33 *of annotations for GO Term ID in Bos Taurus in the EBI GOA database)), Frequency (the proportion of the GO term in the underlying Bos Taurus protein*
34 *annotation database – higher frequency implies more general terms, lower frequency implies more specific terms), Uniqueness (a measure of whether*
35 *the term is an outlier compared to the whole list – calculated by 1-(avg. semantic similarity of term to all other terms) – typically the more unique the*
36 *term the less dispensable) and Dispensability (semantic similarity threshold at which the term was removed from the list and assigned to the cluster).*

GO Term ID	Name	Log(pvalue)	LogSize	Frequency	Uniqueness	Dispensability
GO:0006807	nitrogen compound metabolic process	-3.00	3.89	40.56	0.86	0.00
GO:0007154	cell communication	-1.39	3.72	27.21	1.00	0.00
GO:0007165	signal transduction	-1.43	3.69	25.42	0.86	0.21
GO:0008150	biological_process	-1.66	4.28	100.00	1.00	0.00
GO:0008152	metabolic process	-1.51	3.96	48.11	1.00	0.00
GO:0009893	positive regulation of metabolic process	-1.51	3.49	16.18	0.84	0.59
GO:0009987	cellular process	-1.68	4.20	83.82	1.00	0.00
GO:0010467	gene expression	-1.51	3.25	9.38	0.91	0.23
GO:0019222	regulation of metabolic process	-1.51	3.76	30.24	0.86	0.23
GO:0023052	signaling	-1.39	3.71	26.56	0.86	0.21
GO:0043170	macromolecule metabolic process	-1.51	3.84	36.38	0.86	0.40
GO:0044238	primary metabolic process	-1.85	3.92	43.78	0.86	0.44
GO:0048518	positive regulation of biological process	-1.72	3.71	26.66	0.86	0.21
GO:0048519	negative regulation of biological process	-1.89	3.63	22.18	0.87	0.14
GO:0048522	positive regulation of cellular process	-2.30	3.65	23.43	0.82	0.00
GO:0050789	regulation of biological process	-1.64	4.06	60.16	0.83	0.32
GO:0050794	regulation of cellular process	-2.05	4.03	55.85	0.82	0.20
GO:0050896	response to stimulus	-1.43	3.88	39.84	1.00	0.00

GO:0051239	regulation of multicellular organismal process	-1.89	3.34	11.51	0.89	0.11
GO:0065007	biological regulation	-1.85	4.08	62.67	1.00	0.00
GO:0065008	regulation of biological quality	-1.47	3.37	12.18	0.90	0.10
GO:0071704	organic substance metabolic process	-1.51	3.94	45.74	0.85	0.47

38 Table S8: Differentially expressed lncRNA in the PBLs when CHDL Cases and Control samples of Holstein dairy cows were compared. Identified
39 lncRNA were considered differentially expressed if they had a log2 fold change greater than or equal to 1 or less than or equal to -1 and an adjusted
40 FDR $P < 0.10$. Values include the lncRNA ID, transcript ID the genomic start and end positions, number of exons in the transcript, the class code and
41 base mean log 2 fold change between CHDL case and control, significance p value and p value after correction for multiple testing.

Gene ID	Transcript ID	start	end	Exon number	Class code	tss_id	Base Mean	log2fc	pvalue	padj
XLOC_001685	TCONS_00002626	137718910	137719143	1	u	TSS2090	2.429	-9.23	1.91E-06	0.007
XLOC_002978	TCONS_00004861	77636318	77636556	1	i	TSS3704	1.574	-13.54	2.03E-05	0.038
XLOC_006738	TCONS_00011287	20848315	20848558	1	i	TSS8432	1.165	15.22	3.32E-06	0.010
XLOC_007385	TCONS_00012359	46624336	46624589	1	u	TSS9220	1.859	-17.96	2.05E-08	0.000
XLOC_011112	TCONS_00018769	27040019	27040292	1	i	TSS13966	0.789	-18.22	3.39E-06	0.010
XLOC_011713	TCONS_00019768	71175347	71175624	1	i	TSS14686	1.944	-11.70	7.08E-07	0.003
XLOC_012299	TCONS_00020772	101268674	101268924	1	i	TSS15386	3.757	17.40	7.97E-06	0.017
XLOC_015275	TCONS_00025659	34790285	34790544	2	i	TSS19124	0.411	23.93	8.58E-10	0.000
XLOC_020621	TCONS_00034754	27565252	27565526	1	i	TSS25871	0.624	-21.13	7.17E-08	0.000
XLOC_023405	TCONS_00039374	2045227	2045553	1	i	TSS29383	5.012	15.96	4.73E-05	0.082
XLOC_023452	TCONS_00039443	3453395	3453619	1	u	TSS29440	1.839	-12.48	2.62E-07	0.001
XLOC_024502	TCONS_00041300	64069703	64070006	1	i	TSS30830	1.995	-15.15	7.95E-09	0.000
XLOC_025759	TCONS_00043403	52129892	52130211	1	i	TSS32380	2.839	-13.07	1.24E-08	0.000
XLOC_026191	TCONS_00044187	76479944	76480581	2	u	TSS32910	34.34	-17.86	2.75E-06	0.009
XLOC_026191	TCONS_00044187	76476777	76479741	1	u	TSS32910	34.34	-17.86	2.75E-06	0.009
XLOC_031306	TCONS_00053831	16947487	16947776	1	u	TSS39850	1.784	15.31	8.36E-07	0.003
XLOC_038829	TCONS_00068414	18485731	18486090	1	i	TSS49970	1.802	-12.97	6.91E-06	0.016
XLOC_040747	TCONS_00071609	7162621	7162867	1	u	TSS52304	1.187	17.61	5.96E-06	0.015
XLOC_041028	TCONS_00071994	24446506	24446809	1	u	TSS52642	0.785	16.48	2.41E-05	0.044
XLOC_042990	TCONS_00075162	34198284	34198499	1	u	TSS55095	3.021	15.50	6.46E-05	0.096

XLOC_050950	TCONS_00089040	30431031	30431254	1	<i>i</i>	TSS65397	1.451	-15.70	5.52E-05	0.093
XLOC_050961	TCONS_00089054	31262296	31262744	1	<i>u</i>	TSS65410	1.037	-15.73	6.03E-05	0.093
XLOC_055516	TCONS_00097540	47432273	47432491	1	<i>i</i>	TSS71445	1.463	-12.49	7.10E-06	0.016
XLOC_059563	TCONS_00104303	30218363	30218652	1	<i>u</i>	TSS76580	2.112	-15.88	5.00E-09	0.000
XLOC_060330	TCONS_00105960	6672702	6672965	1	<i>u</i>	TSS77544	0.401	17.01	1.27E-05	0.025
XLOC_062428	TCONS_00109742	114006238	11400650 1	1	<i>u</i>	TSS80378	1.587	15.43	7.10E-05	0.099
XLOC_063128	TCONS_00110966	18826022	18826250	1	<i>u</i>	TSS81263	1.805	-14.70	5.04E-06	0.014
XLOC_063590	TCONS_00111824	41931130	41931362	1	<i>u</i>	TSS81857	2.028	-11.24	1.19E-05	0.024
XLOC_065284	TCONS_00114682	38237407	38237670	1	<i>i</i>	TSS83928	1.258	-14.49	6.75E-05	0.097
XLOC_072236	TCONS_00126680	97062478	97062753	1	<i>u</i>	TSS92811	1.976	-11.91	1.79E-07	0.001
XLOC_073269	TCONS_00128548	25125249	25125502	1	<i>i</i>	TSS94130	1.273	14.18	6.12E-05	0.093
XLOC_077143	TCONS_00135417	45614120	45614601	1	<i>u</i>	TSS99157	1.7	-14.27	5.38E-06	0.014
XLOC_077572	TCONS_00136216	65828736	65829171	1	<i>i</i>	TSS99765	2.132	-13.72	6.11E-05	0.093
XLOC_079207	TCONS_00138856	24947135	24947377	1	<i>u</i>	TSS10179 2	5.292	-10.68	4.26E-10	0.000
XLOC_082800	TCONS_00144472	39625293	39625567	1	<i>i</i>	TSS10615 0	2.216	-14.85	4.46E-13	0.000

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44 Table S9: Statistically significant ($p \leq 0.05$) strong positive (≥ 0.75) and strong negative (≤ -0.75) correlation between long non-coding RNA (lncRNA) and
45 differentially expressed genes in PBL samples.

LncRNA	Protein-coding gene	Correlation value	Adjusted p value
XLOC_050961	<i>ENSBTAG00000025260</i>	0.84	1.4E-05
XLOC_000733	<i>ENSBTAG00000048406</i>	0.67	1.1E-02
XLOC_011112	<i>ENSBTAG00000048406</i>	0.71	4.0E-03
XLOC_041028	<i>ENSBTAG00000048406</i>	0.66	1.4E-02
XLOC_060330	<i>ENSBTAG00000048406</i>	0.80	1.6E-04
XLOC_050961	<i>AMTN</i>	0.85	7.0E-06
XLOC_050961	<i>KRTAP3-3</i>	0.86	3.5E-06
XLOC_050961	<i>ENSBTAG00000051867</i>	0.86	3.5E-06
XLOC_050961	<i>KRTAP27-1</i>	0.86	3.5E-06
XLOC_050961	<i>ENSBTAG00000052798</i>	0.86	3.5E-06
XLOC_050961	<i>ENSBTAG00000054136</i>	0.86	3.5E-06
XLOC_000733	<i>ENSBTAG00000038893</i>	0.89	1.0E-06
XLOC_011112	<i>ENSBTAG00000038893</i>	0.98	3.2E-13
XLOC_015275	<i>ENSBTAG00000038893</i>	0.73	2.3E-03
XLOC_020621	<i>ENSBTAG00000038893</i>	0.91	8.1E-08
XLOC_041028	<i>ENSBTAG00000038893</i>	0.94	4.1E-09
XLOC_060330	<i>ENSBTAG00000038893</i>	0.83	3.4E-05
XLOC_050961	<i>ENSBTAG00000038497</i>	0.74	1.2E-03
XLOC_065284	<i>ENSBTAG00000038497</i>	0.66	1.4E-02
XLOC_077143	<i>ENSBTAG00000038497</i>	0.76	6.3E-04

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48 Table S10: Differentially expressed genes in the foot tissue transcriptome when CHDL Cases and Control samples of Holstein dairy cows were
49 compared. Genes were considered for Gene Set Enrichment Analysis if they had a log2 fold change greater than or equal to 1 or less than or equal to
50 -1, an adjusted FDR $P < 0.10$ and baseMean greater than or equal to 100.

Gene ID	Gene name	Type	Strand	Gene biotype	baseMean	log2fc	Raw P - value	FDR
ENSBTAG00000022373	ENSBTAG00000022373	exon	+	pseudogene	0.710859	-19.474	2.54E-05	0.06
ENSBTAG00000049986	ENSBTAG00000049986	exon	-	protein_coding	0.473533	-19.1057	3.64E-05	0.07
ENSBTAG00000010155	ENSBTAG00000010155	exon	+	protein_coding	29.93062	-3.54263	1.13E-05	0.04
ENSBTAG00000013578	CHI3L2	exon	-	protein_coding	87.66617	-2.97963	7.77E-05	0.07
ENSBTAG00000005260	SPP1	exon	-	protein_coding	66.69538	-2.8955	3.53E-05	0.07
ENSBTAG00000038461	ENSBTAG00000038461	exon	+	protein_coding	46.6398	-2.58261	7.47E-05	0.07
ENSBTAG00000046158	CFB	exon	-	protein_coding	265.5109	-2.38638	7.72E-05	0.07
ENSBTAG00000054974	GRM8	exon	-	protein_coding	71.53197	-2.29535	6.89E-05	0.07
ENSBTAG00000049220	ENSBTAG00000049220	exon	+	protein_coding	396.3796	-2.22345	4.36E-06	0.02
ENSBTAG00000019741	C3AR1	exon	-	protein_coding	39.18599	-2.20847	0.000109	0.10
ENSBTAG00000037634	ENSBTAG00000037634	exon	+	protein_coding	53.33558	-2.18705	7.45E-05	0.07
ENSBTAG00000007153	C1QA	exon	-	protein_coding	131.8391	-2.03939	5.38E-05	0.07
ENSBTAG00000014713	RARRES1	exon	+	protein_coding	156.2307	-1.9032	4.16E-08	0.00
ENSBTAG00000019669	CD163	exon	+	protein_coding	88.28612	-1.89969	1.13E-06	0.01
ENSBTAG00000018137	A2M	exon	+	protein_coding	2352.13	-1.89154	1.14E-05	0.04
ENSBTAG00000007268	F13A1	exon	+	protein_coding	304.7401	-1.51089	2.68E-08	0.00
ENSBTAG00000004305	RGS16	exon	-	protein_coding	138.8612	-1.33051	3.75E-05	0.07
ENSBTAG00000018694	ACSS3	exon	+	protein_coding	219.064	-1.30464	4.34E-05	0.07
ENSBTAG00000012866	THBS4	exon	+	protein_coding	5395.437	-1.09102	6.89E-05	0.07
ENSBTAG00000053420	ENSBTAG00000053420	exon	+	lncRNA	1.788883	16.05713	1.54E-07	0.00
ENSBTAG00000054741	KIR3DL1	exon	-	protein_coding	0.522577	17.56792	0.000124	0.10
ENSBTAG00000007989	FUT9	exon	-	protein_coding	0.522577	17.56792	0.000124	0.10

<i>ENSBTAG00000042477</i>	<i>ENSBTAG00000042477</i>	exon	+	snRNA	0.522577	17.56792	0.000124	0.10
<i>ENSBTAG00000043370</i>	<i>U6</i>	exon	+	snRNA	0.522577	17.56792	0.000124	0.10
<i>ENSBTAG00000015724</i>	<i>BPIFB1</i>	exon	+	protein_coding	0.447923	18.33693	6.20E-05	0.07
<i>ENSBTAG00000048419</i>	<i>ENSBTAG00000048419</i>	exon	+	protein_coding	0.447934	18.33712	6.20E-05	0.07
<i>ENSBTAG00000030532</i>	<i>KNCN</i>	exon	+	protein_coding	0.597231	18.35653	6.02E-05	0.07
<i>ENSBTAG00000048831</i>	<i>ENSBTAG00000048831</i>	exon	-	lncRNA	0.671885	18.43838	5.56E-05	0.07
<i>ENSBTAG00000042498</i>	<i>ENSBTAG00000042498</i>	exon	-	snoRNA	0.746538	18.87873	3.66E-05	0.07
<i>ENSBTAG00000042118</i>	<i>7SK</i>	exon	-	misc_RNA	0.764604	19.30533	2.45E-05	0.06
<i>ENSBTAG00000050223</i>	<i>bta-mir-2285cu</i>	exon	-	miRNA	1.063543	19.6649	1.72E-05	0.05

52 Table S11: Gene Set Enrichment Analysis (GSEA) results based on the differential expression analysis of the foot tissue transcriptome when CHDL
53 Case compared to Control samples in Holstein dairy cows. The Table presents the significant biological processes (BP) enriched among the
54 differentially expressed genes. Columns include the Ontology and Gene Ontology ID, Ontology description, the number of genes in the set (setSize),
55 the normalized enrichment score (NES), the FDRadjusted p-value, and gene set names. Positive NES values represent enrichment in upregulated
56 genes, while negative NES values indicate enrichment in downregulated genes.

ONTOLOGY	ID	Description	setSize	NES	FDR	Gene set enriched
BP	GO:0001525	angiogenesis	3	-1.69	0.006	ENSBTAG000000018223/ENSBTAG000000021434
BP	GO:0035239	tube morphogenesis	3	-1.69	0.006	ENSBTAG000000018223/ENSBTAG000000021434
BP	GO:0048514	blood vessel morphogenesis	3	-1.69	0.006	ENSBTAG000000018223/ENSBTAG000000021434
BP	GO:0002684	positive regulation of immune system process	6	-1.76	0.010	ENSBTAG000000001729/ENSBTAG000000007153/ENSBTAG000000046158/ENSBTAG000000021434
BP	GO:0051246	regulation of protein metabolic process	5	-1.64	0.018	ENSBTAG000000009717/ENSBTAG000000013125/ENSBTAG000000001729/ENSBTAG000000018223
BP	GO:0001775	cell activation	5	-1.62	0.022	ENSBTAG000000009717/ENSBTAG000000001729/ENSBTAG000000007153/ENSBTAG000000021434
BP	GO:0045321	leukocyte activation	5	-1.62	0.022	ENSBTAG000000009717/ENSBTAG000000001729/ENSBTAG000000007153/ENSBTAG000000021434
BP	GO:0034097	response to cytokine	5	-1.61	0.022	ENSBTAG000000018223/ENSBTAG000000021434
BP	GO:0001932	regulation of protein phosphorylation	3	-1.57	0.024	ENSBTAG000000001729/ENSBTAG000000018223

BP	GO:0019220	regulation of phosphate metabolic process	3	-1.57	0.024	ENSBTAG00000001729/ENSBTAG00000018223
BP	GO:0031399	regulation of protein modification process	3	-1.57	0.024	ENSBTAG00000001729/ENSBTAG00000018223
BP	GO:0042325	regulation of phosphorylation	3	-1.57	0.024	ENSBTAG00000001729/ENSBTAG00000018223
BP	GO:0051174	regulation of phosphorus metabolic process	3	-1.57	0.024	ENSBTAG00000001729/ENSBTAG00000018223
BP	GO:0071345	cellular response to cytokine stimulus	4	-1.65	0.025	ENSBTAG00000018223/ENSBTAG00000021434
BP	GO:0022008	neurogenesis	4	-1.65	0.025	ENSBTAG00000001729/ENSBTAG00000007153/ ENSBTAG00000021434
BP	GO:0035295	tube development	4	-1.65	0.025	ENSBTAG00000018223/ENSBTAG00000021434
BP	GO:0001568	blood vessel development	4	-1.62	0.030	ENSBTAG00000014614/ENSBTAG00000018223/ ENSBTAG00000021434
BP	GO:0001944	vasculature development	4	-1.62	0.030	ENSBTAG00000014614/ENSBTAG00000018223/ ENSBTAG00000021434
BP	GO:0048646	anatomical structure formation involved in morphogenesis	4	-1.62	0.030	ENSBTAG00000014614/ENSBTAG00000018223/ ENSBTAG00000021434
BP	GO:0072359	circulatory system development	4	-1.62	0.030	ENSBTAG00000014614/ENSBTAG00000018223/ ENSBTAG00000021434
BP	GO:0022407	regulation of cell-cell adhesion	3	-1.55	0.031	ENSBTAG00000013125/ENSBTAG00000001729
BP	GO:0030155	regulation of cell adhesion	3	-1.55	0.031	ENSBTAG00000013125/ENSBTAG00000001729

BP	GO:0051247	positive regulation of protein metabolic process	3	-1.55	0.031	<i>ENSBTAG000000013125/ENSBTAG00000018223</i>
BP	GO:0035556	intracellular signal transduction	6	-1.61	0.037	<i>ENSBTAG00000007687/ENSBTAG00000013125/ENSBTAG00000001729/ENSBTAG00000018223/ENSBTAG000000021434</i>
BP	GO:0050790	regulation of catalytic activity	5	-1.58	0.046	<i>ENSBTAG000000009717/ENSBTAG00000013125/ENSBTAG00000001729/ENSBTAG00000018223</i>
BP	GO:0048584	positive regulation of response to stimulus	10	-1.53	0.047	<i>ENSBTAG000000004840/ENSBTAG00000015032/ENSBTAG00000007687/ENSBTAG00000013125/ENSBTAG00000007153/ENSBTAG000000046158/ENSBTAG00000018223/ENSBTAG00000021434</i>
BP	GO:0006793	phosphorus metabolic process	5	-1.57	0.048	<i>ENSBTAG00000017411/ENSBTAG00000013125/ENSBTAG00000001729/ENSBTAG00000018223</i>
BP	GO:0006796	phosphate-containing compound metabolic process	5	-1.57	0.048	<i>ENSBTAG00000017411/ENSBTAG00000013125/ENSBTAG00000001729/ENSBTAG00000018223</i>
BP	GO:0016310	phosphorylation	5	-1.57	0.048	<i>ENSBTAG00000017411/ENSBTAG00000013125/ENSBTAG00000001729/ENSBTAG00000018223</i>

58 *Table S12: Gene ontology enrichment results summary by Revigo based on the differential expression analysis of the foot tissue transcriptome when*
59 *CHDL Cases compared to Control samples*
60 *Columns include log(pvalue) of GO enrichment of term, LogSize (the Log10(number of annotations for GO*
61 *Term ID in Bos Taurus in the EBI GOA database)), Frequency (the proportion of the GO term in the underlying Bos Taurus protein annotation database*
62 *– higher frequency implies more general terms, lower frequency implies more specific terms), Uniqueness (a measure of whether the term is an outlier*
63 *compared to the whole list – calculated by 1-(avg. semantic similarity of term to all other terms) – typically the more unique the term the less*
dispensable) and Dispensability (semantic similarity threshold at which the term was removed from the list and assigned to the cluster).

GO Term ID	Name	Log(pvalue)	LogSize	Frequency	Uniqueness	Dispensability
GO:0001775	cell activation	-1.66	2.73	2.79	0.85	0.23
GO:0001932	regulation of protein phosphorylation	-1.62	2.93	4.42	0.71	0.06
GO:0019220	regulation of phosphate metabolic process	-1.62	3.02	5.46	0.76	0.93
GO:0042325	regulation of phosphorylation	-1.62	2.97	4.91	0.76	0.90
GO:0001944	vasculature development	-1.52	2.60	2.07	0.67	0.49
GO:0002684	positive regulation of immune system process	-2.00	2.89	4.04	0.91	0.00
GO:0006793	phosphorus metabolic process	-1.32	3.23	8.94	0.96	0.24
GO:0016310	phosphorylation	-1.32	2.93	4.45	0.92	0.01
GO:0006796	phosphate-containing compound metabolic process	-1.32	3.23	8.81	0.92	0.79
GO:0022008	neurogenesis	-1.60	2.98	5.04	0.70	0.55
GO:0022407	regulation of cell-cell adhesion	-1.51	2.55	1.85	0.94	0.07
GO:0030155	regulation of cell adhesion	-1.51	2.75	2.96	0.94	0.08
GO:0031399	regulation of protein modification process	-1.62	3.04	5.78	0.79	0.67
GO:0051247	positive regulation of protein metabolic process	-1.51	3.04	5.70	0.76	0.70
GO:0034097	response to cytokine	-1.66	2.82	3.44	0.94	0.62
GO:0035239	tube morphogenesis	-2.22	2.70	2.60	0.67	0.00
GO:0001525	angiogenesis	-2.22	2.36	1.18	0.63	0.90

GO:0001568	blood vessel development	-1.52	2.58	1.98	0.65	0.94
GO:0048514	blood vessel morphogenesis	-2.22	2.49	1.61	0.62	0.84
GO:0035295	tube development	-1.60	2.82	3.43	0.73	0.46
GO:0035556	intracellular signal transduction	-1.43	3.09	6.48	0.90	0.24
GO:0045321	leukocyte activation	-1.66	2.66	2.40	0.86	0.23
GO:0048584	positive regulation of response to stimulus	-1.33	3.23	8.85	0.90	0.34
GO:0048646	anatomical structure formation involved in morphogenesis	-1.52	2.85	3.71	0.75	0.63
GO:0050790	regulation of catalytic activity	-1.34	3.15	7.40	0.95	0.06
GO:0051174	regulation of phosphorus metabolic process	-1.62	3.02	5.46	0.83	0.36
GO:0051246	regulation of protein metabolic process	-1.74	3.30	10.54	0.82	0.38
GO:0071345	cellular response to cytokine stimulus	-1.60	2.77	3.09	0.94	0.00
GO:0072359	circulatory system development	-1.52	2.82	3.46	0.72	0.48

65 Table S13: Differentially expressed lncRNA in the foot tissue when CHDL Cases and Control samples of Holstein dairy cows were compared. Identified
66 lncRNA were considered differentially expressed if they had a log2 fold change greater than or equal to 1 or less than or equal to -1 and an adjusted
67 FDR $P < 0.10$. Values include the lncRNA ID, transcript ID the genomic start and end positions, number of exons in the transcript, the class code and
68 base mean log 2 fold change between CHDL case and control, significance p value and p value after correction for multiple testing.

Gene ID	Transcript ID	start	end	Exon number	Class code	tss_id	Base Mean	log2fc	pvalue	padj
XLOC_000293	TCONS_00000507	47584813	47585116	1	u	TSS405	0.74	19.28	2.52E-05	0.015
XLOC_000383	TCONS_00000689	59719542	59719835	1	u	TSS538	0.45	18.27	6.58E-05	0.023
XLOC_000438	TCONS_00000768	67342845	67343156	1	u	TSS608	0.82	18.20	6.89E-05	0.023
XLOC_002835	TCONS_00005559	66759314	66759616	1	u	TSS4061	2.34	20.06	1.14E-05	0.015
XLOC_003414	TCONS_00006756	22293772	22294020	1	i	TSS4897	2.95	19.83	2.35E-09	0.000
XLOC_005470	TCONS_00010621	28978280	28978582	1	u	TSS7703	1.39	17.61	0.000119	0.038
XLOC_005471	TCONS_00010622	28984777	28985014	1	u	TSS7704	2.02	19.33	2.40E-05	0.015
XLOC_005678	TCONS_00010967	58645164	58645508	1	u	TSS7952	1.70	19.58	1.86E-05	0.015
XLOC_006273	TCONS_00012161	13818362	13818649	1	u	TSS8765	0.60	18.98	3.38E-05	0.016
XLOC_006918	TCONS_00013503	34624356	34624666	1	i	TSS9730	2.00	19.03	3.06E-05	0.016
XLOC_007102	TCONS_00013804	80564872	80565140	1	u	TSS9959	2.02	19.37	2.29E-05	0.015
XLOC_009264	TCONS_00017888	38509989	38510278	1	u	TSS12972	1.04	19.58	1.86E-05	0.015
XLOC_010130	TCONS_00019463	1281964	1282261	1	u	TSS14121	1.05	19.33	2.35E-05	0.015
XLOC_013169	TCONS_00025071	69337103	69337402	1	u	TSS18295	0.75	18.68	4.44E-05	0.018
XLOC_016705	TCONS_00031928	9847247	9847547	1	i	TSS23343	2.07	19.86	1.40E-05	0.015
XLOC_018904	TCONS_00036413	10030655	10030999	1	u	TSS26511	1.50	19.58	1.83E-05	0.015
XLOC_019175	TCONS_00036937	49233949	49234227	1	u	TSS26905	0.75	18.68	4.44E-05	0.018
XLOC_020380	TCONS_00039272	84179061	84179379	1	u	TSS28622	1.28	19.47	2.08E-05	0.015
XLOC_021051	TCONS_00040516	18067935	18068225	1	u	TSS29525	1.20	19.27	2.48E-05	0.015
XLOC_021230	TCONS_00040867	48671403	48671712	1	u	TSS29779	1.55	18.82	3.97E-05	0.018
XLOC_023340	TCONS_00044868	34625916	34626173	1	u	TSS32752	0.67	19.17	2.77E-05	0.015
XLOC_023830	TCONS_00045857	15138167	15138440	1	i	TSS33495	0.67	19.17	2.77E-05	0.015

<i>XLOC_024584</i>	<i>TCONS_00047365</i>	24980254	24980527	1	u	TSS34587	1.20	19.27	2.48E-05	0.015
<i>XLOC_029120</i>	<i>TCONS_00056310</i>	33636179	33636499	1	u	TSS41099	0.90	18.97	3.32E-05	0.016
<i>XLOC_029340</i>	<i>TCONS_00056733</i>	19422702	19422932	1	i	TSS41408	0.65	19.17	2.80E-05	0.015
<i>XLOC_029777</i>	<i>TCONS_00057677</i>	31210841	31211058	1	u	TSS42048	0.45	18.27	6.58E-05	0.023
<i>XLOC_031565</i>	<i>TCONS_00061238</i>	39930824	39931176	1	u	TSS44598	1.50	19.58	1.83E-05	0.015
<i>XLOC_034665</i>	<i>TCONS_00067307</i>	48133886	48134157	1	u	TSS48914	1.05	19.70	1.64E-05	0.015
<i>XLOC_038031</i>	<i>TCONS_00073640</i>	40425643	40425916	1	i	TSS53576	0.60	17.21	0.000169	0.052
<i>XLOC_038968</i>	<i>TCONS_00075364</i>	73139585	73139863	1	u	TSS54859	1.41	-18.61	5.74E-05	0.022
<i>XLOC_039169</i>	<i>TCONS_00075774</i>	1.04E+08	1.04E+08	1	i	TSS55127	0.75	18.68	4.44E-05	0.018
<i>XLOC_041614</i>	<i>TCONS_00080528</i>	94624676	94624945	1	u	TSS58544	1.36	20.03	1.18E-05	0.015
<i>XLOC_042004</i>	<i>TCONS_00081264</i>	49196068	49196368	1	u	TSS59102	1.12	19.21	2.63E-05	0.015
<i>XLOC_042121</i>	<i>TCONS_00081481</i>	63342609	63342945	1	i	TSS59270	0.82	18.20	6.89E-05	0.023
<i>XLOC_043157</i>	<i>TCONS_00083411</i>	84008871	84009081	1	i	TSS60685	0.45	18.27	6.58E-05	0.023

70 Table S14: Statistically significant ($p \leq 0.05$) strong positive (≥ 0.75) and strong negative (≤ -0.75) correlation between lncRNA and differentially
71 expressed genes in foot tissue samples.

LncRNA	Protein-coding gene	Correlation value	Adjusted p value
XLOC_000293	ENSBTAG00000042498	0.89	1.1E-04
XLOC_000293	ENSBTAG00000048831	0.89	1.1E-04
XLOC_000293	FUT9	0.89	1.1E-04
XLOC_000293	ENSBTAG00000042477	0.89	1.1E-04
XLOC_000293	KIR3DL1	0.89	1.1E-04
XLOC_000293	BPIFB1	0.89	1.1E-04
XLOC_000293	ENSBTAG00000048419	0.89	1.1E-04
XLOC_000293	KNCN	0.89	1.1E-04
XLOC_000438	ENSBTAG00000042498	1.00	0.0E+00
XLOC_000438	ENSBTAG00000048831	1.00	0.0E+00
XLOC_000438	FUT9	1.00	8.2E-91
XLOC_000438	ENSBTAG00000042477	1.00	8.2E-91
XLOC_000438	KIR3DL1	1.00	8.2E-91

XLOC_000438	BPIFB1	1.00	0.0E+00
XLOC_000438	ENSBTAG00000048419	1.00	0.0E+00
XLOC_000438	KNCN	1.00	0.0E+00
XLOC_002835	ENSBTAG00000053420	0.74	8.8E-03
XLOC_002835	ENSBTAG00000042498	0.65	4.0E-02
XLOC_002835	ENSBTAG00000048831	0.65	4.0E-02
XLOC_002835	FUT9	0.65	4.0E-02
XLOC_002835	ENSBTAG00000042477	0.65	4.0E-02
LncRNA	Protein-coding gene	Correlation value	Adjusted p value
XLOC_002835	KIR3DL1	0.65	4.0E-02
XLOC_002835	BPIFB1	0.65	4.0E-02
XLOC_002835	ENSBTAG00000048419	0.65	4.0E-02
XLOC_002835	KNCN	0.65	4.0E-02
XLOC_005470	RGS16	-0.75	7.3E-03

XLOC_00547 0	ENSBTAG0000004249 8	0.64	4.8E-02
XLOC_00547 0	ENSBTAG0000004883 1	0.64	4.8E-02
XLOC_00547 0	FUT9	0.64	4.8E-02
XLOC_00547 0	ENSBTAG0000004247 7	0.64	4.8E-02
XLOC_00547 0	KIR3DL1	0.64	4.8E-02
XLOC_00547 0	BPIFB1	0.64	4.8E-02
XLOC_00547 0	ENSBTAG0000004841 9	0.64	4.8E-02
XLOC_00547 0	KNCN	0.64	4.8E-02
XLOC_00627 3	ENSBTAG0000004249 8	0.95	1.7E-06
XLOC_00627 3	ENSBTAG0000004883 1	0.95	1.7E-06
XLOC_00627 3	FUT9	0.95	1.7E-06
XLOC_00627 3	ENSBTAG0000004247 7	0.95	1.7E-06
XLOC_00627 3	KIR3DL1	0.95	1.7E-06
XLOC_00627 3	BPIFB1	0.95	1.7E-06
XLOC_00627 3	ENSBTAG0000004841 9	0.95	1.7E-06
XLOC_00627 3	KNCN	0.95	1.7E-06

XLOC_00710 2	C3AR1	-0.64	4.9E-02
XLOC_00710 2	RGS16	-0.76	6.7E-03
XLOC_00926 4	ENSBTAG0000004249 8	0.82	1.4E-03
XLOC_00926 4	ENSBTAG0000004883 1	0.82	1.4E-03
XLOC_00926 4	FUT9	0.82	1.4E-03
XLOC_00926 4	ENSBTAG0000004247 7	0.82	1.4E-03
XLOC_00926 4	KIR3DL1	0.82	1.4E-03
XLOC_00926 4	BPIFB1	0.82	1.4E-03
XLOC_00926 4	ENSBTAG0000004841 9	0.82	1.4E-03
XLOC_00926 4	KNCN	0.82	1.4E-03
XLOC_01013 0	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_01013 0	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_01013 0	FUT9	1.00	1.5E-91
XLOC_01013 0	ENSBTAG0000004247 7	1.00	1.5E-91
XLOC_01013 0	KIR3DL1	1.00	1.5E-91
XLOC_01013 0	BPIFB1	1.00	0.0E+00

XLOC_01013 0	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_01013 0	KNCN	1.00	0.0E+00
XLOC_01316 9	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_01316 9	ENSBTAG0000004883 1	1.00	2.9E-90
XLOC_01316 9	FUT9	1.00	0.0E+00
XLOC_01316 9	ENSBTAG0000004247 7	1.00	0.0E+00
XLOC_01316 9	KIR3DL1	1.00	0.0E+00
XLOC_01316 9	BPIFB1	1.00	0.0E+00
XLOC_01316 9	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_01316 9	KNCN	1.00	0.0E+00
XLOC_01890 4	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_01890 4	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_01890 4	FUT9	1.00	0.0E+00
XLOC_01890 4	ENSBTAG0000004247 7	1.00	0.0E+00
XLOC_01890 4	KIR3DL1	1.00	0.0E+00
XLOC_01890 4	BPIFB1	1.00	2.9E-90

XLOC_01890 4	ENSBTAG0000004841 9	1.00	1.5E-91
XLOC_01890 4	KNCN	1.00	0.0E+00
XLOC_02334 0	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_02334 0	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_02334 0	FUT9	1.00	1.5E-91
XLOC_02334 0	ENSBTAG0000004247 7	1.00	1.5E-91
XLOC_02334 0	KIR3DL1	1.00	1.5E-91
XLOC_02334 0	BPIFB1	1.00	2.9E-94
XLOC_02334 0	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_02334 0	KNCN	1.00	2.9E-94
XLOC_03156 5	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_03156 5	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_03156 5	FUT9	1.00	0.0E+00
XLOC_03156 5	ENSBTAG0000004247 7	1.00	0.0E+00
XLOC_03156 5	KIR3DL1	1.00	0.0E+00
XLOC_03156 5	BPIFB1	1.00	2.9E-90

XLOC_03156 5	ENSBTAG0000004841 9	1.00	1.5E-91
XLOC_03156 5	KNCN	1.00	0.0E+00
XLOC_03896 8	ENSBTAG0000004998 6	0.83	1.0E-03
XLOC_03896 8	ENSBTAG0000002237 3	0.83	1.0E-03
XLOC_04200 4	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_04200 4	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_04200 4	FUT9	1.00	2.9E-94
XLOC_04200 4	ENSBTAG0000004247 7	1.00	2.9E-94
XLOC_04200 4	KIR3DL1	1.00	2.9E-94
XLOC_04200 4	BPIFB1	1.00	0.0E+00
XLOC_04200 4	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_04200 4	KNCN	1.00	0.0E+00
XLOC_02458 4	ENSBTAG0000004249 8	1.00	0.0E+00
XLOC_02458 4	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_02458 4	FUT9	1.00	1.6E-92
XLOC_02458 4	ENSBTAG0000004247 7	1.00	1.6E-92

XLOC_02458 4	KIR3DL1	1.00	1.6E-92
XLOC_02458 4	BPIFB1	1.00	0.0E+00
XLOC_02458 4	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_02458 4	KNCN	1.00	0.0E+00
XLOC_00038 3	ENSBTAG0000004249 8	1.00	8.2E-91
XLOC_00038 3	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_00038 3	FUT9	1.00	1.5E-91
XLOC_00038 3	ENSBTAG0000004247 7	1.00	1.5E-91
XLOC_00038 3	KIR3DL1	1.00	1.5E-91
XLOC_00038 3	BPIFB1	1.00	1.6E-92
XLOC_00038 3	ENSBTAG0000004841 9	1.00	0.0E+00
XLOC_00038 3	KNCN	1.00	0.0E+00
XLOC_02977 7	ENSBTAG0000004249 8	1.00	8.7E-90
XLOC_02977 7	ENSBTAG0000004883 1	1.00	0.0E+00
XLOC_02977 7	FUT9	1.00	2.9E-90
XLOC_02977 7	ENSBTAG0000004247 7	1.00	2.9E-90

XLOC_02977 7	<i>KIR3DL1</i>	1.00	2.9E-90
XLOC_02977 7	<i>BPIFB1</i>	1.00	1.5E-91

XLOC_02977 7	<i>ENSBTAG0000004841</i> 9	1.00	0.0E+00
XLOC_02977 7	<i>KNCN</i>	1.00	0.0E+00

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73

74 *Table S15: Genes with identified Cis eQTLs in PBL samples and the number of cis eQTLs identified for*
75 *each gene.*

Gene stable ID	Gene name	Number of cis eQTLs
ENSBTAG00000013802	DAB1	202
ENSBTAG00000003774	C8A	198
ENSBTAG00000007217	TMEM94	185
ENSBTAG00000017704	ABCG2	101
ENSBTAG00000008032	ACTR3B	75
ENSBTAG00000014302	TOMM70	44
ENSBTAG00000001008	PRF1	38
ENSBTAG00000026836	ABI3BP	22
ENSBTAG00000016363	TFG	3
ENSBTAG00000004742	RUNX1	3
ENSBTAG00000017811	SLC39A9	2
ENSBTAG00000023384	CBR1	2
ENSBTAG00000003484	CZIB	1
ENSBTAG00000008576	PLEKHD1	1
ENSBTAG00000013961	MYSM1	1
ENSBTAG00000001998	ST6GALNAC1	1

76

77 *Table S16: Genes with identified cis eQTLs in foot tissue samples and the number of cis acting SNPs*
78 *identified for each gene.*

Gene stable ID	Gene name	Number of cis eQTLs
ENSBTAG00000013802	DAB1	496
ENSBTAG00000030913	MX1	221
ENSBTAG00000021253	PRDM15	121
ENSBTAG00000024195	LDLRAD1	112
ENSBTAG00000003484	CZIB	111
ENSBTAG00000016363	TFG	101
ENSBTAG00000006625	NDC1	91
ENSBTAG00000000202	SLC25A19	85
ENSBTAG00000046447	Novel cattle gene	69
ENSBTAG00000052302	Novel cattle gene	55
ENSBTAG00000010758	SMIM5	54
ENSBTAG00000021276	TFF3	31
ENSBTAG00000000736	LRP8	17
ENSBTAG00000047101	CCDC177	17
ENSBTAG00000009002	Novel cattle gene	12
ENSBTAG00000021582	NCAPG	9

ENSBTAG00000003857	SUSD6	7
ENSBTAG00000043015	U6	6
ENSBTAG00000053000	<i>Novel cattle gene</i>	4
ENSBTAG00000011973	DCAF16	3
ENSBTAG00000021259	C2CD2	2
ENSBTAG00000019198	LRRC42	2
ENSBTAG00000013321	<i>Novel cattle gene</i>	1
ENSBTAG00000026836	ABI3BP	1
ENSBTAG00000016932	SEN7	1
ENSBTAG00000000394	BACE2	1
ENSBTAG00000009132	TMPRSS2	1
ENSBTAG00000030814	TFF2	1
ENSBTAG00000004742	RUNX1	1
ENSBTAG00000000470	IBSP	1
ENSBTAG00000011596	SRSF5	1
ENSBTAG00000044273	U6	1
ENSBTAG00000016243	RBL2	1
ENSBTAG00000022006	TRIM65	1
ENSBTAG00000011713	<i>Novel cattle gene</i>	1
ENSBTAG00000007217	TMEM94	1
ENSBTAG00000021497	CDH23	1
ENSBTAG00000010888	VSIR	1

Table S17: Genes identified with differentially methylated bases that were also identified as differentially expressed by RNA-seq analysis, detailing the number of differentially methylated bases (DM), the distance of each differentially methylated base to the gene's transcription start site (TSS) and the percentage difference in methylation in CHDL samples compared to the controls.

ID	Gene name	No. DM bases	Distance to TSS (bp)	MethDiff (%)
ENSBTAG00000007268	F13A1	9	21667, 105784, -29868, -26780, 38775, 13222, 24116, -5766, -99	31.79, 30.23, 26.67, -32.19, -36.47, -37.70, -43.24, -48.12, -59.49, -73.90
ENSBTAG00000025260	WAP four-disulfide core domain protein 18-like	2	14909, 14818	42.11, 42.65
ENSBTAG00000019297	GFRA1	2	437785, 327939	-43.89, 26.71
ENSBTAG00000011182	PDLIM1	1	9490	-53.21
ENSBTAG00000007153	C1QA	1	-751	-28.11

* The first gene (ENSBTAG00000007268) was differentially expressed only in the foot tissue sample, the rest in PBL samples.

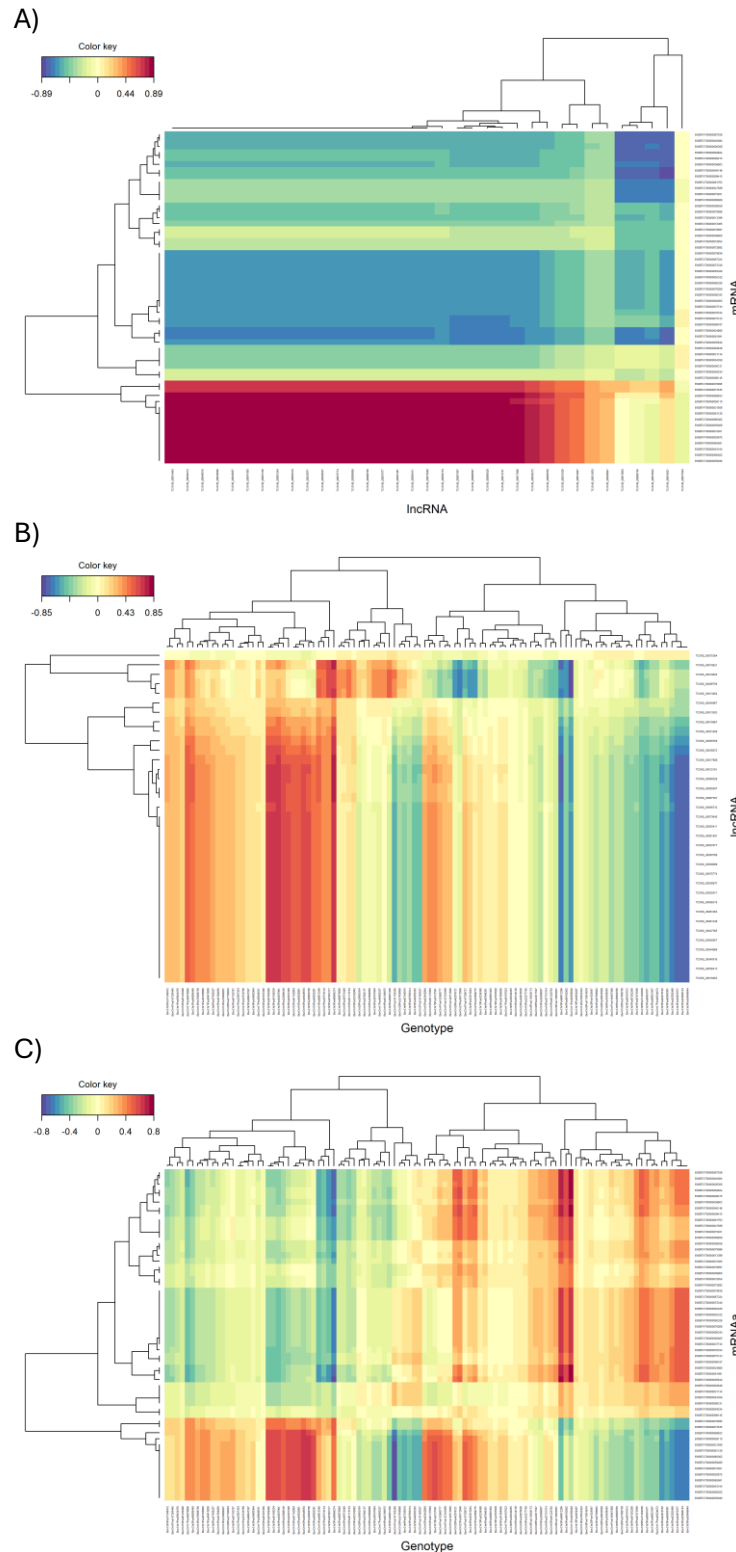


Figure S1: Partial Least Squares Discriminant Analysis (PLS-DA) analysis results based on the foot tissue multiomics data. Heatmaps presenting the correlation between different features (mRNA, lncRNA and SNP genotype) based on the PLSDA analysis. A) correlations between mRNA and lncRNA B) correlations between lncRNA and SNP genotype C) correlations between mRNA and SNP genotype. Size of correlation and direction is indicated by the colour key in each graph.

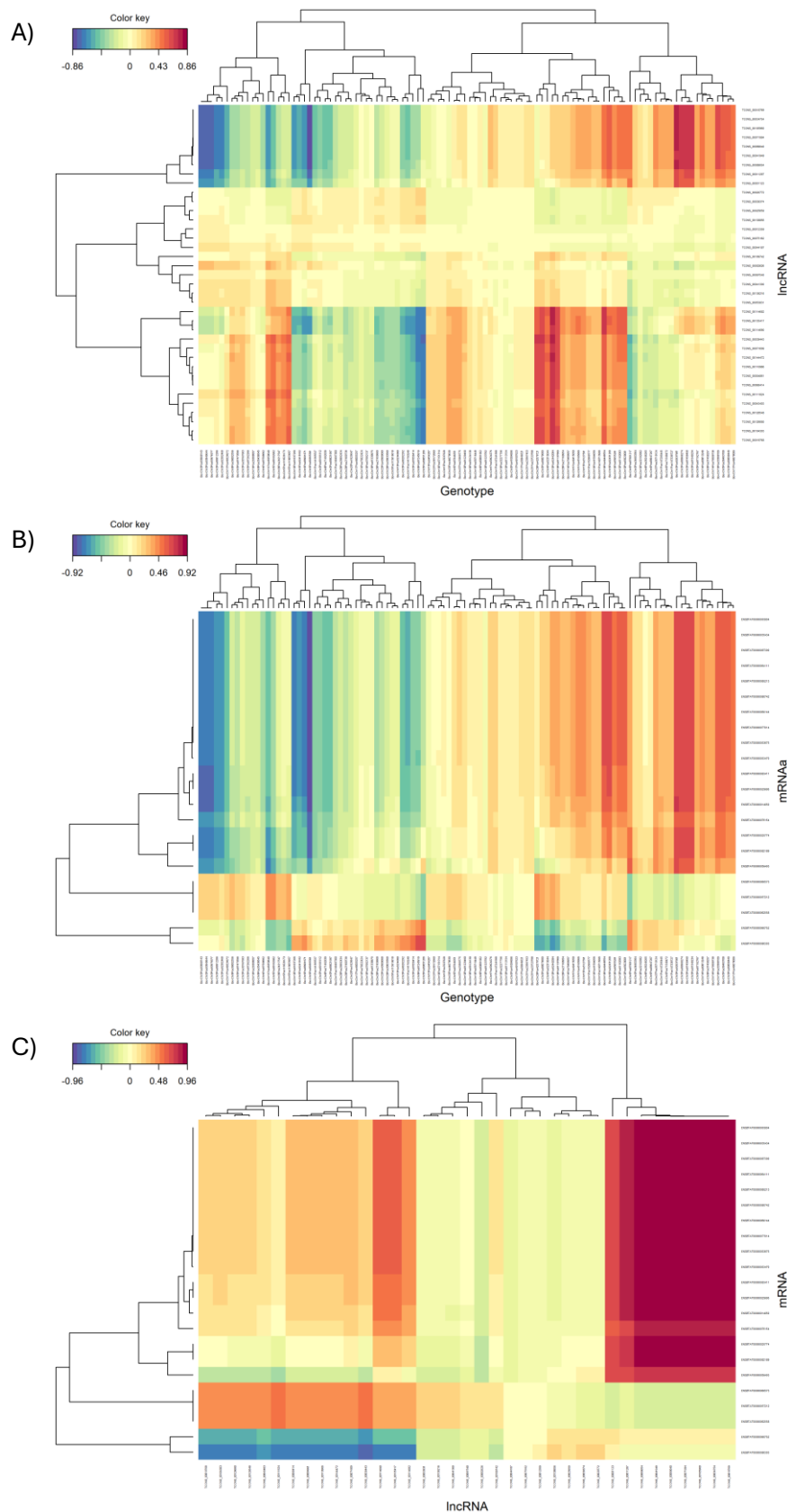
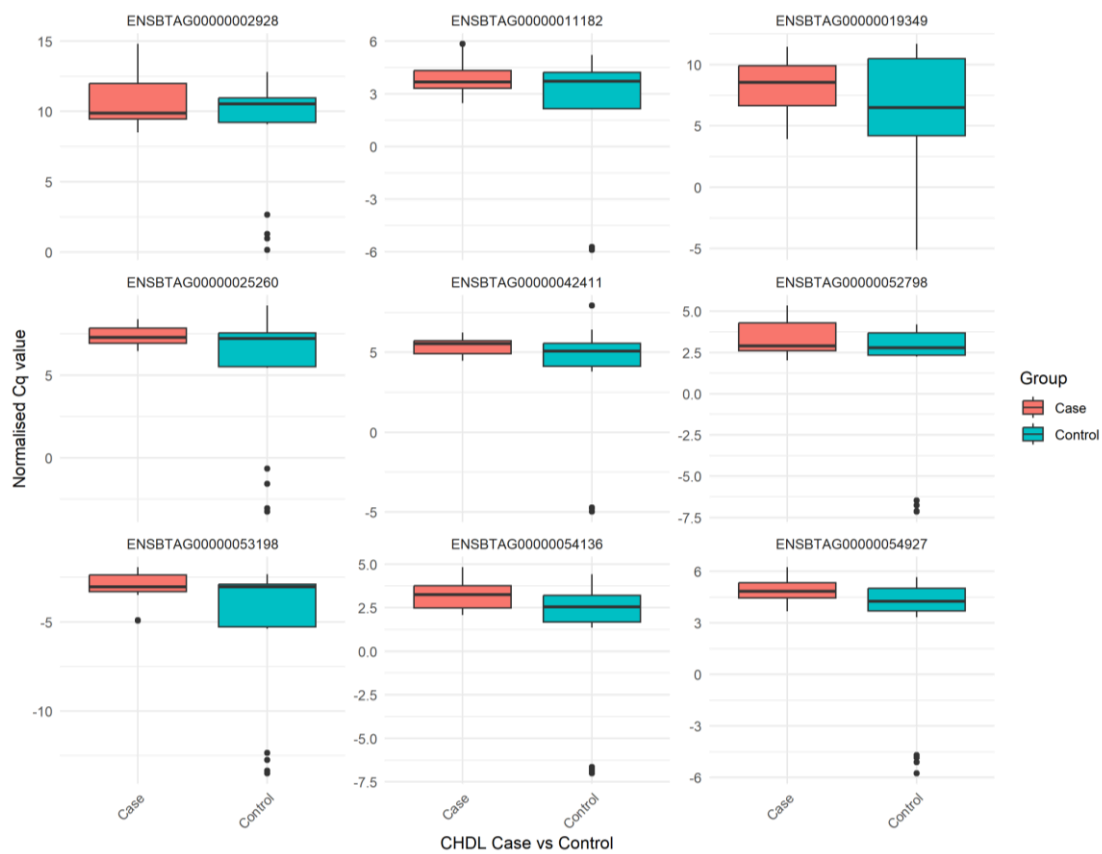


Figure S2: Heatmaps of correlation between features from the peripheral blood leukocyte (PBL) Partial Least Squares Discriminant Analysis (PLS-DA) analysis (mRNA, IncRNA and SNP genotype). A) IncRNA and SNP genotype B) mRNA and SNP genotype C) mRNA and IncRNA. Correlation is indicated by the colour key in each graph.

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156

157 *Figure S3: Validation of RNA-Seq analysis in PBL results using RT-qPCR. Box plots of RT-qPCR*
158 *Cq value normalised using RSP9 housekeeping gene identified as statistically significantly*
159 *different between CHDL case and control samples in peripheral blood leukocyte (PBL) RNA.*
160 *(ENSBTAG00000002928: AMTN, ENSBTAG00000011182: PDLIM1, ENSBTAG00000019349:*
161 *SLC6A4, ENSBTAG00000025260, ENSBTAG00000042411: U6, ENSBTAG00000052798,*
162 *ENSBTAG00000053198, ENSBTAG00000054136, ENSBTAG00000054927: KRTAP3-3).*

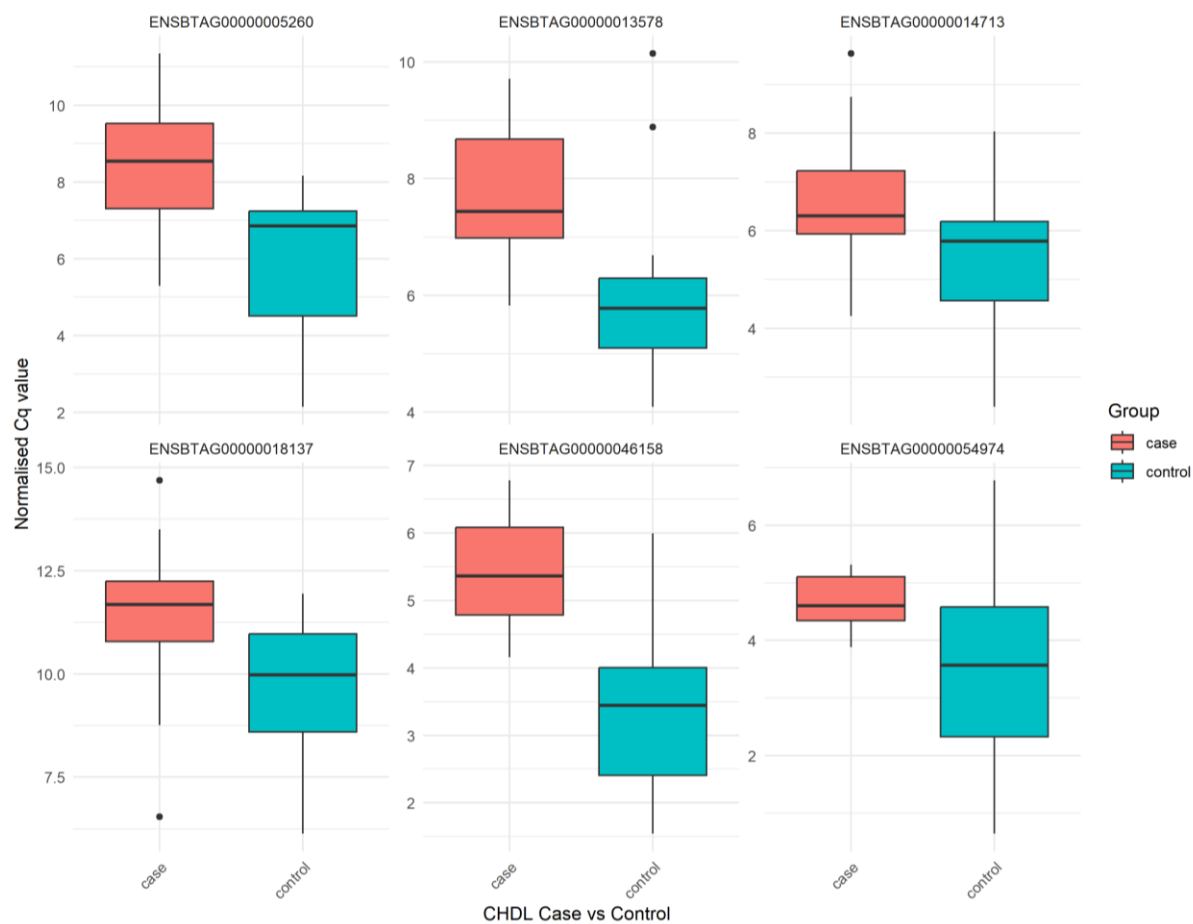
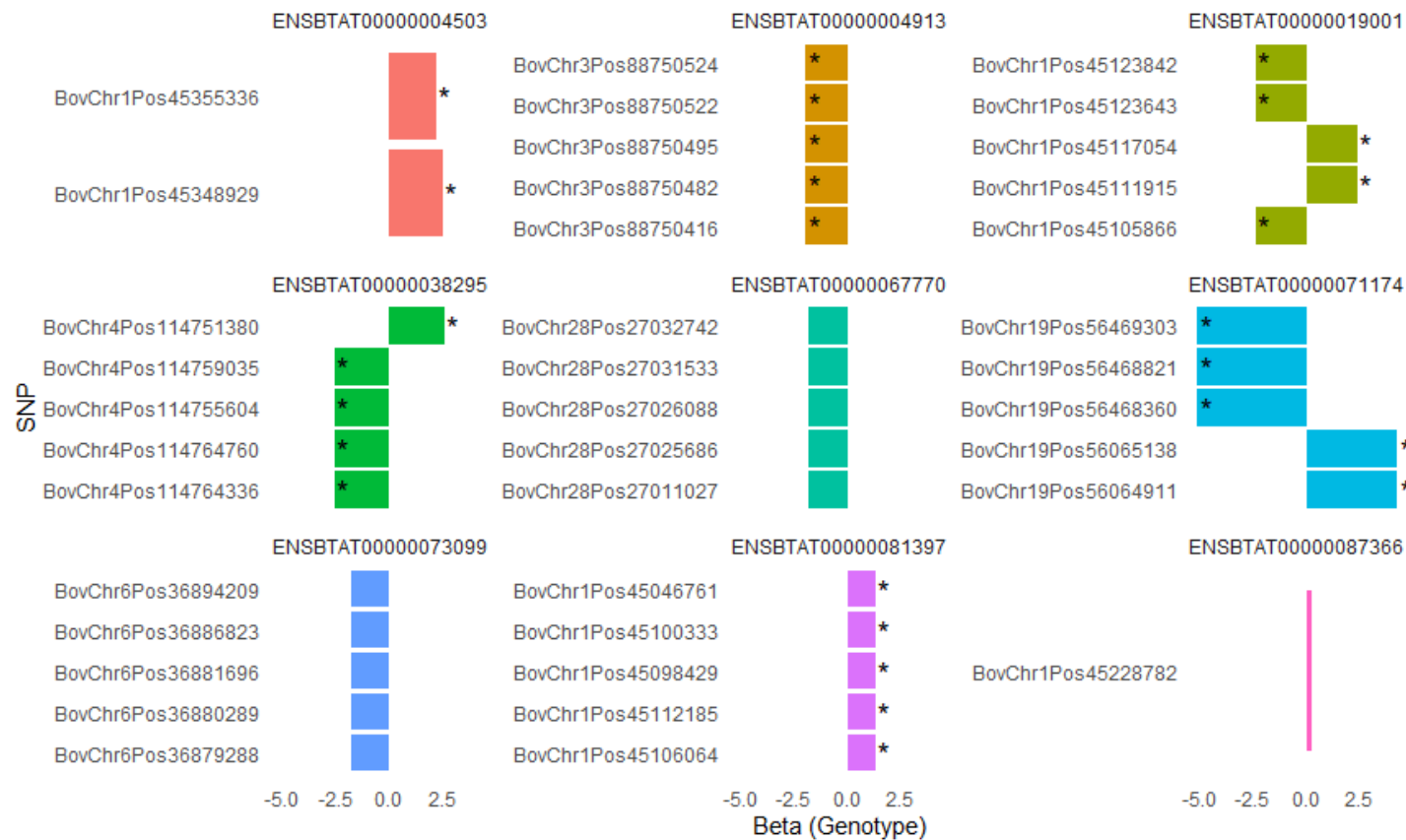
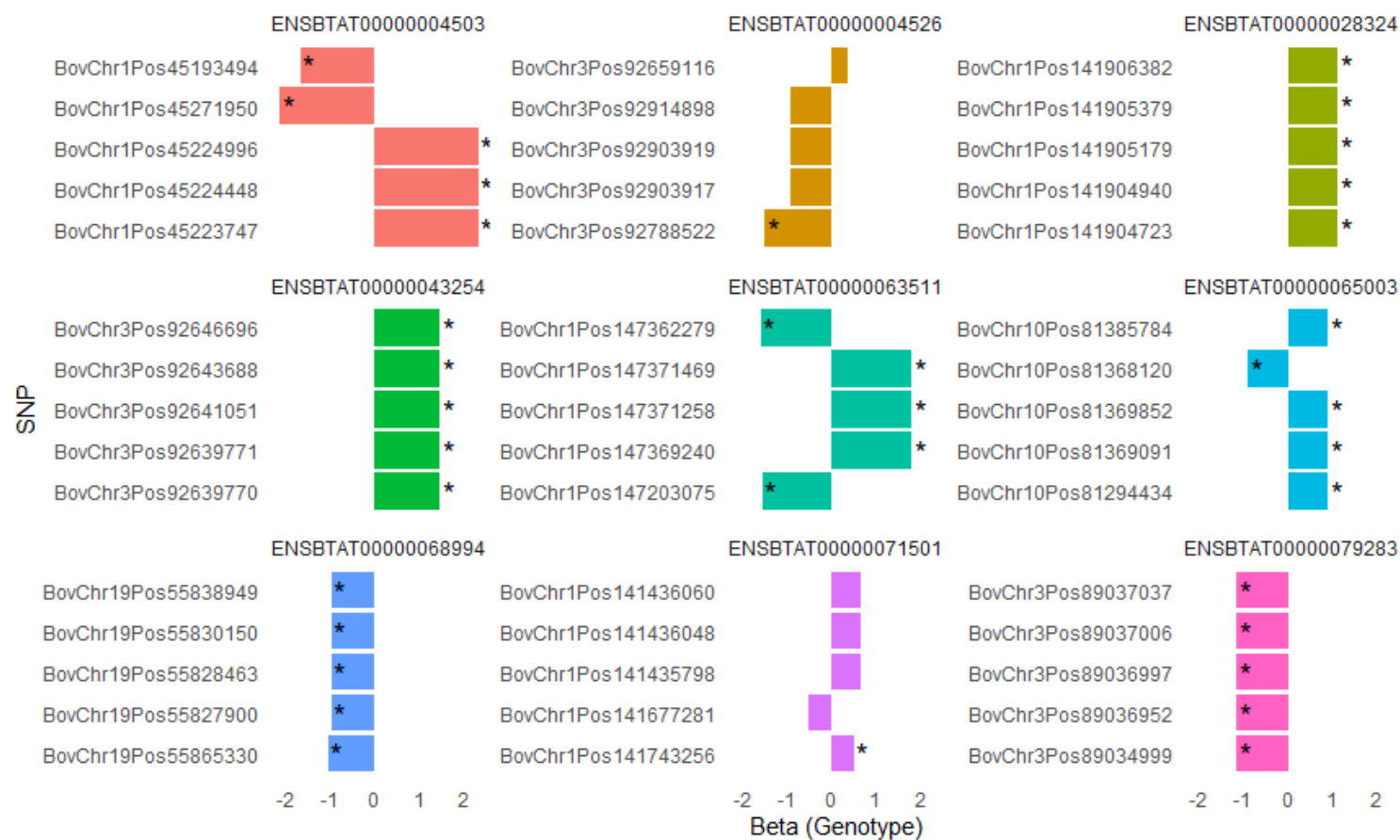


Figure S4: Validation of RNA-Seq analysis in foot tissue results using RT-qPCR. Box plots of RT-qPCR Cq value normalised using RSP9 housekeeping gene identified as statistically significantly different between CHDL case and control samples in foot tissue RNA. (ENSBTAG00000005260 : SPP1, ENSBTAG00000013578: CHI3L2, ENSBTAG00000014713: RARRES1, ENSBTAG00000018137: A2M, ENSBTAG00000046158: CFB, ENSBTAG00000054974: GRM8).



169

170 Figure S5: Bar plot of the top genotype effects of identified significant PBL cis eQTLs on RT-qPCR Cq value by target transcript. Corresponding to the
 171 genes *TFG*, *C8A*, *TOMM70*, *ACTR3B*, *PRF1*, *TMEM94*, *ABCG2*, *ABI3BP* (both ENSBTAT00000081397 and ENSBTAT00000087366) (from left to right, top
 172 to bottom). The x axis (Beta value) represents the effect size of the SNP genotype on gene expression with * indicating statistical significance.



173

174 Figure S6: Bar plot of the top genotype effects of identified significant foot tissue cis eQTLs on RT-qPCR Cq value by target transcript. Corresponding
 175 to the genes *TFG*, *CZIB*, *PRDM15*, *LDLRAD1*, *ENSBTAG000000046447*, *CCDC177*, *SLC25A19*, *MX1* and *DAB1* (from left to right, top to bottom). The x
 176 axis (Beta value) represents the effect size of the SNP genotype on gene expression with * indicating statistical significance.

177



Figure S7: Western blot image of protein lysate from PBL samples with anti-bovine cytokeratin antibody staining at ~35kDa in all seven samples demonstrating the presence of cytokeratin including KRTAP3-3 and KRTAP27-1 .

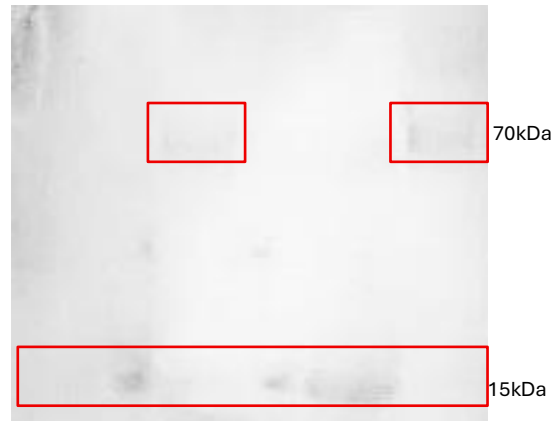


Figure S8: Western blot image of protein lysate from foot tissue samples with anti-bovine CD163 antibody staining at ~15kDa in three samples and possibly ~70kDa in two samples indicating the presence of CD163 epitope fragments.

Table S18: Primer sequences used for RT-qPCR validation of identified differentially expressed genes, detailed is the primer sequence (5' to 3') and the target gene ID. Forward and reverse primer pairs are indicated by _F or _R after the gene ID.

Primer sequence (5' to 3')	Primer name
GCACAAGGATGGTACTGCCT	ENSBTAG00000046158_F
CAGTGAAACAGTGTGCAGCC	ENSBTAG00000046158_R
CCAACCAGGGTCTCATGCAT	ENSBTAG00000018137_F
CGGAGAATCTTTGGGCACCT	ENSBTAG00000025260_R
ATTTGTGGTTGCAGCTGCTG	ENSBTAG00000002928_R
TCATCGCCTTCTACATCGCC	ENSBTAG00000019349_F
GTGTTCCAGGAGTTCTCGCA	ENSBTAG00000019349_R
CCTAGCTTTTCTGGGCAGCT	ENSBTAG00000014713_F
TTTCAGTGGTTTGCTGGGGT	ENSBTAG00000014713_R
TCGTTGTCATTGGGCACGTA	ENSBTAG00000053198_F
TCCTGGAGCAGCAGAACAAG	ENSBTAG00000052798_F
AGGGAACACTTCACAAATTGCA	ENSBTAG00000042411_R

Table S19: Primer sequences used for RT-qPCR validation of cis eQTLs, detailed is the primer sequence (5' to 3') and the target gene ID. Forward and reverse primer pairs are indicated by _F or _R after the gene ID. The identification of the eQTL in either the foot tissue or PBL analysis is indicated in the eQTL column.

Primer sequence (5' to 3')	Primer name	eQTL
GATGCTTTCGGCGAGCAGAG	ENSBTAT00000079283_F	FT
AGCTGCGGAAACCTCATCAA	ENSBTAT00000079283_R	FT
GGCCTTCTATTCAAAGAAGCACA	ENSBTAT00000004503_F	Both
TGCATTGGTGCCCTCATTCT	ENSBTAT00000004503_R	Both
TGAAGATATGGAGTCCAAGAGCA	ENSBTAT00000071501_F	FT
GCCTCAGCACAAGAGGACAT	ENSBTAT00000071501_R	FT
AGCACTTCGCGTAGTTCCAA	ENSBTAT00000028324_F	FT
ATTCAACGGAGAGGGGCAAG	ENSBTAT00000028324_R	FT
TTTGACGAATGCGGGTCAAG	ENSBTAT00000068994_F	FT
TGCAAGGAGTTGCTGGGTAG	ENSBTAT00000068994_R	FT
ATGGACAGTGTGGCACTGAA	ENSBTAT00000004526_F	FT
ACTTCTCGTTGTCAATGGAGTTTTC	ENSBTAT00000004526_R	FT
AGAAGGATGCAAACGAGAGAGAA	ENSBTAT00000063511_F	FT
GAGATTGCAGTCAGCCAGCG	ENSBTAT00000063511_R	FT
GAGGCGTTGTTGAAAGGTCG	ENSBTAT00000065003_F	FT
GCTCCACCAACTGCTCGTAA	ENSBTAT00000065003_R	FT
GAGGCCCTTGTCGAGGTGT	ENSBTAT00000043254_F	FT
CCCCACAGTTGTTGATCCCA	ENSBTAT00000043254_R	FT
GCCTGGGGTAACCATACAGG	ENSBTAT00000004913_F	PBL
TGGCTTGGTCCCAGACATTC	ENSBTAT00000004913_R	PBL
CAGATGAGCAAGTCTCACGC	ENSBTAT00000071174_F	PBL
CTGAGAGGACACGGCTTCTG	ENSBTAT00000071174_R	PBL
CGTTTTCTTGGGACGCTAC	ENSBTAT00000019001_F	PBL
GGCCATAACGAGTGCCTCC	ENSBTAT00000019001_R	PBL
GTTCAATTATCCCTTCATGTATCGCC	ENSBTAT00000038295_F	PBL
GCCTCCCGAAAGAACGACA	ENSBTAT00000038295_R	PBL
CCACCGTCAAACCTTCCCACT	ENSBTAT00000081397_F	PBL
ACAGCAGATTCAAGGCACGG	ENSBTAT00000081397_R	PBL

Supplementary methods

Calculation of CHDL score

CHDL score was based on sole haemorrhage (SH), sole ulcer (SU) and white line disease (WLD) scores combined into one single CHDL phenotype. CHDL score for individual animals was calculated as follows:

(1). Re-scaling SU score to account for the fact that SU develops from evolved severe SH cases. In the re-scaling, SU score of 0, 1, 2, or 3 were re-scaled to 0, 4, 5, or 6, respectively.

(2) Combining SH (0/1/2/3) and SU (0/4/5/6) scores to derive a Sole-lesion score for each claw: If the SU score was not 0, the Sole-lesion score was set equal to the SU score; If SU score was equal to 0, the Sole-lesion score was set equal to the SH score.

(3) Calculating the average of Sole-lesion score across all measured claws of each animal (i.e., mean-Sole-lesion)

(4) Calculating the average of WLD score across all measured claws of each animal (i.e., mean-WLD)

(5) The CHDL score of each animal is the sum of its mean-Sole-lesion (from Step 3) and mean-WLD (from Step 4)

Each animal had four repeated measurements of CHDL score across time which were averaged to obtain the final CHDL score for each animal.

Blood sample collection and peripheral blood lymphocytes isolation

Blood samples for isolation of bovine peripheral blood lymphocytes (PBLs) were collected from the jugular vein of the 24 cattle selected into 6 of 10ml VECUETTE® EDTA tubes (Greiner Bio-one, Kremsmünster, Austria). PBLs were isolated by erythrocyte lysis [1], briefly, 4ml of anti-coagulated blood was added to 40ml ammonium chloride lysing solution (8.29g NH₄Cl, 1g KHCO₃ and 37mg Na₂EDTA in 1L distilled water, pH7.2), and incubated at room temperature for 10min. PBLs were harvested by centrifugation and washed three times with 40ml of phosphate buffered saline (PBS)

with 1% bovine serum albumin solution. PBLs were counted by trypan blue dye exclusion revealing >90% cell viability. Isolated PBLs were resuspended in 350µL BufferRLT from the RNeasy Mini kit (Qiagen, Hilden, Germany) at 10⁶cells/mL and stored at -80°C. Concurrently, 10⁶cells/mL of isolated PBL were resuspended in 200µL PBS. Then, 40µL Proteinase K from the DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) and 20µL of RNase A (20 mg/mL; PureLink™, Invitrogen™, Massachusetts, United States) were added, and incubated at room temperature for 2 minutes. 300µL of BufferAL from the DNeasy Blood & Tissue Kits was added and incubated at 56°C for 2 hours followed by 70°C for 15 minutes. The resulting lysate was stored at -80°C.

Foot tissue biopsy collection

Biopsy specimens of foot tissue were obtained from 14 of the selected cows. For case animals, biopsies were performed on the foot bearing the lesion. The foot chosen for control animals was selected to reflect the matched case, or if a minor lesion was present, the opposite hind claw was chosen. Before biopsy specimen collection, the foot was washed with water to remove manure/debris. An initial scrub with Chlorhexidine was performed, followed by the application of local anaesthesia using 20ml Adrenacaine (Norbrook Inc. Newry, Northern Ireland). The anaesthetic was allowed to take effect and the presence of anaesthesia tested, followed by a final scrub with surgical spirit and gauze. A tourniquet was placed around the proximal metatarsus, and a ~7mm hole was drilled in the horn at the dorsal hoof wall with 1mm of horn left behind at the biopsy site using a drill and dremmel. A 6-mm biopsy punch with a maximum coring depth of 6mm (Integra Miltex; Integra life Science Corporation, York, PA, USA) was used to biopsy the remaining horn layer and part of the corium, and the sample was carefully removed with a scalpel blade and dressing forceps. The sample was placed in a labelled cryovial, adding 1ml Allprotect tissue reagent (Qiagen, Hilden, Germany). The biopsies in Allprotect were stored at 4°C overnight and then -20°C. After biopsy sampling, lavage with saline was performed at the biopsy site followed by application of topical antibiotic spray and bandage. Finally, 20mls of Ketofen (Zoetis Canada Inc., Kirkland, Quebec, Canada) was administered via Intramuscular injection in the neck.

RT-qPCR

RT-qPCR was performed to validate RNA-Seq and eQTL analysis results, in duplicate, for all 24 PBL and 14 foot tissue RNA samples. Reverse transcription was performed in a 20µL reaction using 300ng of extracted RNA using LunaScript RT SuperMix (5X) (New England Biolabs[®]) following the manufacturer's instructions including thermocycler conditions, a single cycle of each: annealing 25°C 2 minutes, cDNA synthesis 55°C 10 minutes and heat inactivation 95°C 1 minute. cDNA was stored at -20°C and used for qPCR analysis. Primers for qPCR analysis were designed using ThermoFisher Oligo perfect (<https://www.thermofisher.com/uk/en/home/life-science/oligonucleotides-primers-probes-genes/custom-dna-oligos/oligo-design-tools.html>) and produced using Invitrogen custom primers. Primer sequences are provided in Supplementary Table S18-19. The qPCR 20µL reaction mix was made according to the Luna Universal qPCR mastermix (New England Biolabs[®]) manufacturer specifications. Thermocycler conditions were adapted from manufacturer specifications: initial denaturation - 95°C 60 seconds, denaturation - 95°C 15 seconds, annealing (and data acquisition) – 60°C 30 seconds, extension – 72°C 15 seconds, additional data acquisition – 85°C, 5 seconds, melt curve – 60-95°C. Denaturation to additional data acquisition cycles x40. Samples were normalised using the housekeeping gene Radial spoke head protein 9 (*RSP9*) before statistical analysis. A CHDL case vs control statistical analysis of Cq values was performed; initially a shapiro-wilks normality test was performed and then a T-test for each gene investigated. For the validation of target genes from the eQTL analyses a linear model was performed to investigate if there was a significant association between the SNP genotype and Cq value.

Western blot analysis

Residual PBL samples were lysed for western blot analysis and foot tissue homogenised by bead beating as previously described in the main methods section. Protein concentration for each sample was measured using a Qubit4 Fluorometer (Invitrogen, USA) and the Qubit Protein Assay kit (Invitrogen, USA) according to manufacturer's instructions. 10µL of PageRuler pre-stained NIR protein

ladder (P1219) (ThermoScientific, USA) was run per gel. The same amount of protein per sample was combined with Novex bolt 4X LDS sample buffer (Invitrogen) to a final concentration of 1X and heated to 90°C for 10 minutes before being loaded into the wells of the gel. NuPAGE Bis-Tris 4–12% (Invitrogen, USA) gels were used for SDS-PAGE in MES buffer NuPAGE™ (Invitrogen, USA), run for 30 minutes at 180V. Gels were then transferred to a PVDF Membrane (Immobilon, Germany) using NuPAGE™ Transfer Buffer (Invitrogen, USA) at 20V for 1 hour. Both SDS-PAGE and transfer were conducted in an Invitrogen Mini gel tank according to manufacturer's instructions. Membranes were blocked with 5% milk powder (Aptamil 1) in 1X PBS with 0.1% Tween20 (ThermoScientific, USA) overnight at 4°C. Blots were incubated with primary antibody for 1 hour at room temperature. Foot tissue blot: CD163 antibody | 2A10/11 Mouse anti Pig CD163 (BioRad) 1:200 diltution. PBL blot: Cytokeratin antibody | C-11 (BioRad) 1:500 diltution. Blots were washed thoroughly with 1X PBS with 0.1% Tween20 then incubated in secondary antibody Goat anti Mouse IgG (H/L):HRP (Multi Species Adsorbed) (BioRad) 1:1000 dilution for 1 hour at room temperature before being washed thoroughly with 1X PBS with 0.1% Tween20 and signal detected by HRP substrate (Millipore Immobilon forte western HRP substrate, USA). Blots were imaged using an Invitrogen iBright1500 and quantification of signal performed using ImageJ (<https://imagej.net/ij/index.html>).

References:

1. Werling, D., et al., *Analysis of the phenotype and phagocytic activity of monocytes/macrophages from cattle infected with the bovine leukaemia virus*. Veterinary immunology and immunopathology, 1998. **62**(3): p. 185-195.